The Effect of Enzymatic Degradation on PLA Home-Compostability to Reduce Microplastic Pollution

Alyson Dai

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This study aims to examine and monitor the rate of disintegration and home-compostability of PLA (Polylactic acid) samples with various amounts of enzymatic additives to understand potential solutions for reducing microplastic pollution. PLA is a bioplastic made of natural materials like cornstarch or sugar cane. PLA, despite its affordability, excellent functional properties, and performance, is only industrial compostable. In this study PLA samples consist of 0%, 2.5% and 5% enzymes for microbial degradation were tested. PHA (Polyhydroxyalkanoates), a microbially biodegradable plastic, was included as a control. Different forms of these plastics, such as lids, foam, and sheets were tested in a home compost. The samples were checked and measured every three weeks throughout six months. It was discovered that the higher the microbial enzyme concentration in PLA, the faster the degradation rate, although high crystallinity may slow down the disintegration. In PLA samples with 5% concentration of enzymes, on average about 85% of the PLA lids by weight were disintegrated and over 92% of the carbon in the PLA was converted to CO_2 after 6 months, some of the lids were even completely degraded; this is comparable to PHA which was 100% decomposed within 24 weeks, while the neat PLA without embedded enzymes had no measurable degradation and fragmentation. It was concluded that embedded enzymes can significantly accelerate the biodegradation rate of PLA in backyard compost. This research shows that PLA with microbial enzymes can be potentially home compostable, thus providing a considerable solution to the problem of non-biodegradable plastic pollution in the natural environment.

Keywords: Microplastic Pollution, PLA, enzyme, home-compostable

Introduction

Microplastic pollution is a major environmental issue in the U.S. and across the rest of the world. The UN Environment Program estimated that over 400 million tons of plastic are produced every year for use in a wide variety of applications[1]. At least 14 million tons of plastic end up in the ocean yearly, and plastic makes up about 80% of all marine debris found from surface waters to deep-sea sediments[1,2]. Microplastics are defined as plastic particles less than five millimeters (0.2 inches) in diameter that are not biodegradable resulting from commercial product development, manufacturing, and the breakdown or photodegradation of larger plastics[3]. Like plastic items of any size, microplastics do not readily break down into harmless molecules. As a pollutant, microplastics or microbeads can be harmful to the environment and have many negative effects on wildlife and animal health[3,4]. However, plastics have been and are still used extensively around the world because of their many undeniable benefits and advantages. Given the wide use of plastics in everyday life, banning all plastic usage and production would therefore be very illogical, particularly without suitable alternatives or replacements.

The biodegradability of a plastic refers to the ability of a polymer to self-degrade into natural substances (biomass, water, and carbon dioxide) within a certain, reasonable amount of time that other fossil-fuel-based plastics cannot. While compostable plastic also biodegrades, it is specifically designed to be processed in either home or industrial composting facilities[5,6]. Polylactic Acid (PLA), with the chemical formula of (C3H4O2)n, is derived from renewable resources, such as corn, potato, and sugarcane. It is chemically polymerized from sugars (lactic acid) via bacterial fermentation and is a semi-crystalline, hydrophilic, and polar polyester. PLA as a biodegradable plastic has great usage within the plastics industry, while still providing an environmentally friendly alternative to fossil-fuel-based plastics to fight microplastic pollution.

However, while PLA is considered a biodegradable plastic, it is only industrially compostable and degraded in high temperatures, and humid environments containing relevant microorganisms[5], in other words, PLA is not biodegradable in just any environment. The rates at which the fragmentation, hydrolysis, and ultimate biodegradation of PLA proceed in the environment depend on PLA properties like crystallinity and environmental variables such as water availability, temperature, and available microorganisms. In the environment of industrial composting facilities, temperature and water content are optimized, so that

the hydrolysis and following biodegradation of PLA are fast. In a natural environment, the time to reach complete hydrolysis and eventual biodegradation is assumed to range from months to years, depending primarily on temperature and the presence of water[5]. This makes PLA incompatible or practically non-compostable with home composting, soil conditions, and other nature environments, limiting PLA's practical benefits, thus causing its accumulation in landfills and contribution to plastics pollution. In the process of biodegradation of PLA, it undergoes two stages: first, hydrolysis or oxidation into monomers and oligomers, and then finally metabolization by microorganisms that produce CO_2 and H_2O[7]. For PLA to degrade fast, the degradation process must be catalyzed by heat and humid conditions as well as relevant microorganisms that possess PLA degrading mechanism because of various enzymes in their organisms. Therefore, accelerating biodegradation rate of PLA under nature ambient conditions through microbially or enzymatically catalyzed reactions is important to understand and urgently desired to investigate.

A number of enzymes play an important role in the depolymerization of PLA, including carboxylesterase, esterase, lipase, and serine protease (i.e. Proteinase K), which have been investigated for degradation of PLA[8,9]. The biodegradation of PLA is reliant on Proteinase K which was identified as the most important enzyme involved in catalyzing the hydrolytic degradation of PLA[9]. However, the PLA-degrading enzymes are not available in most environment except on very rare occasions[7]. More challenge is that the thermostability and activity of enzymes is typically below the glass transition temperature (50-60^{o}C) of PLA, limiting their suitability for the processing of PLA articles[10]. Further, enzymes are typically too big to diffuse into the polymer matrix[10], thus the enzyme catalyzed hydrolysis of PLA is limited to the surface area where microbes are nearby. The research aims to solve these challenges, seeking and selecting compatible PLA-degrading enzymes, investigating the applicability and effectiveness of embedding the enzymes in PLA matrix to degrade PLA far beyond its surface level within the PLA structure at a macromolecular level. It was hypothesized that enzyme-embedded PLA could show immense potential in achieving faster biodegradation for single-use food packaging with the effectiveness of properly selected and embedded enzymes. The goals of this research were to test the hypothesis, determine, and possibly improve the rate of biodegradation and home compostability of PLA through enzymatic degradation in backyard home compost. The following experiments were conducted based on ASTM (American Society for Testing and Materials) and ISO (International Organization for Standards) standards for biodegradation and composting specification, as well as test methods in a simulated natural environment. The outcomes of this research could pave the way for developing innovative solutions for sustainable and home-compostable food packaging, offering significant benefits for environmental protection and public health while reducing microplastic pollution.

Methodology

Testing Standards

Composting is a two-step process, which includes 1) Disintegration – the moisture and heat in the compost break down the polymer chains creating smaller fragments. 2) Biodegradation – microorganisms in compost and soil consume and metabolize the smaller polymer fragments. The end result of composting is carbon dioxide (CO_2), water (H_2O) and humus, a soil nutrient. The EN 13432 or ASTM D6400 Standard requires samples 1) Biodegradation – to convert 90 percent of the carbon in the material to CO_2 after 180 days (6 months) at 58°C; 2) disintegration – 90% of material is smaller than 2 mm after 90 days (3 months) at 40-70°C, depending on the standard for industrial composting. A modification of EN13432 by TUV Austria also requires tests performed at 20-30°C over time frames that are twice as long as those in the original tests for home composting. The following Table 1 summarizes the biodegradation standards and conditions, which served as the experiment and test reference of this research project.

	Industrial Composting	**Home Composting**
Standard	EN 13432:2000 ISO 14855 ASTM D6400 ASTM D6868	EN 17427 NF T51-800
Test Temperature	58 ˚C ± 2 ˚C	28 ˚C ± 2 ˚C
Biodegradation Time	6 months	12 months
Disintegration Time	12 weeks	6 months

Table 1 Overview of Biodegradation and Composting Standards and Test Conditions

Sample Preparation and Experimental Set-up

The purpose of this experiment is to increase PLA's biodegradation rate for home compostability by adding and blending selected enzymes in the biochemical formulation. Evanesto® enzymes from Carbiolice are selected for this experiment. They were mixed and blended with Ingeo PLA 2003D from NatureWork at different concentration levels of loading percentage (0%, 2.5%, and 5%) by weight. Home compostable TÜV certified Danimer PHA samples were included for comparison as a control. In sample preparation, Ingeo PLA 2003D or Danimer PHA was first pre-dried to a moisture level of 250ppm (parts per million) or less (400 ppm for PHA) in a dryer. Then the PLA or PHA granules and the encapsulated enzyme Evanesto additives were loaded at designated percentages (0%, 2.5%, 5%)

and mixed in a blender, which fed the mixture into an extruder hopper along a heated cylinder by a rotating screw with speed of 700rpm, melted at around 175°C (150°C for PHA), compressed and plasticized into a homogenous molten mass under pressure of around 725 psi, which was further pressurized, conveyed and extruded to the sheet die. The extrusion residence time was 4 min 30s. The extrudate was then cooled via chill rolls and transported to a slitting and winding station to produce sheets at a thickness of about 17 mils. Thermoforming is a low-pressure, low-temperature process that treats plastic as a rubbery or elastic solid. In this process, the extruded PLA sheet was first heated at a temperature range of 70°C–110°C and then pressurized air was applied to shape the sheet under vacuum to the contours of a lid forming tool. Three-dimensional lids with embedded Evanesto® enzyme were then created as a result.

The types of samples tested include neat (100%) PLA lids, neat PLA 2 x 2" sheets, PLA with 2.5% enzyme lids, PLA with 2.5% enzyme 2 x 2" sheets, PLA with 5% enzyme lids, PLA with 5% enzyme 2 x 2" sheets. All the samples were weighted initially by a scale (model: Scout Pro SP_20_2 from OHAUS with an accuracy of 0.01g) and summarized in Table 2. The testing conditions in compost were set to have humidity above 55% and pH being around 8 under ambient temperatures ranges from 30°C to 0°C during the test period from June to December. The average temperature over the test period is below 30°C representing the natural environment. The moisture and pH were measured by Luster Leaf digital moisture meter and electronic soil tester. The preparation of composting inoculum followed ISO 14855 by mixing 50% organic compost soil amendment with 50% garden soil from Home Depot adding some vegetable waste and fruit peels as well as leaves, grass clippings and yard trimmings etc. Water was sprayed into the inoculum to reach the required humidity. The activity of the inoculum and microbes was checked during the test by means of the compostable control samples (PHA lids and sheets) and by measuring the carbon dioxide evolution in the blank vessels with the cellulose reference, which should be degraded by 70% or more at the end of the test according to ISO 14855. Composting is a biological process during which naturally occurring microorganisms, bacteria, and insects in the test environment break down organic materials that are biodegradable and compostable. The listed samples were mixed and buried in the inoculum in the backyard compost bin under above-mentioned temperature, moisture, and pH conditions. The samples were checked every 3 weeks over 6 months from June to December, and each time the samples were carefully examined for changes in appearance and surface, size and thickness, cracks and breakages, etc. In addition to visual examination, a Jiusion Digital Microscope was used to check if any micro crazing. Observations were then documented in a notebook, with pictures taken to record the progress of the disintegration. Dimensions were measured using a ruler (cm/inch), and thickness was measured by a magnetic wall thickness (mil) gauge Olympus (model: Magna-Mike 8600). Weights (g) were measured at the beginning and end of the composting (post-washed and dried) using the same scale. ECHO Instruments Respirometry System – Respirometer ER Series™ was utilized for measuring CO_2 production in the process of biodegradation to determine the biodegradability of the samples. The system measured CO_2 concentration in flow through the samples under controlled conditions based on ISO 14855. Flow, temperature, pressure, and humidity were also measured continuously. The associated software automatically calculates CO_2 production and biodegradation percentage. Crystallization and melting temperatures as well as heat flow of the samples were measured by Differential Scanning Calorimetry (DSC, TA model Q400), degradation on-set temperature was measured by Thermal Gravimetric Analysis (TGA, TA model 5500).

The hypothesis of this experiment is that adding proper enzymes into PLA will increase the biodegradation rate of PLA, thus resulting in its home compostability and providing a viable solution for microplastic pollution. Variables within this experiment included composition (enzyme concentration), crystallinity, density and weight, size, and shape of the samples, with the control or comparing variable being PHA due to its high biodegradability and compostability in soil, freshwater, and marine environments, as certified by TUV.

Experimental Results

The following photos in Figure 1-4 showed the testing progress and result of PLA with various concentrations of Evanesto® enzymes added in different samples of PLA. Figure 1 compared neat (100%) PLA sheets and lids with the control sample, neat PHA sheet, during the composting. PHA sheet was completely degraded and disappeared at the end of composting or 24 weeks, while neat PLA samples without the addition of the enzymes, both sheets and lids, stayed unchanged over the course with no sign of any fragmentation, cracking, or brittleness.

Figures 2 and 3 are pictured composting results for both lids and sheet samples made from PLA with 2.5% enzymes and PLA with 5% enzymes respectively. Compared to neat PLA samples shown in Fig.1, PLA with 2.5% enzymes started cracking and fragmenting slowly, while PLA with 5% enzymes broke and disintegrated much faster. At the end of the 24-week test, over 85-90% of the PLA with 5% enzymes samples were degraded and disappeared.while neat PLA without enzymes had no disintegration and fragmentation at all over the same test period, this indicated that the Evanesto® enzymes helped increase the ability and rate of PLA biodegradation in home composter and natural environments. It is expected that with further optimization of enzyme concentration as well as process improvement in future work, the modified enzyme-embedded PLA can be disintegrated faster enough to meet home-compostable requirement (>90% disintegration). Similarly, all the lids were degraded faster than

	Initial Drinking Lid Weight (g)						Initial 2"x2" sheet weight (g), 17 mil			
Samples	Neat PLA unfoamed	PLA + 2.5% Enzyme unfoamed	PLA + 5% Enzyme unfoamed	PLA + 5% Enzyme foamed	Neat PLA foamed	PHA unfoamed	Neat PLA unfoamed	PLA + 2.5% Enzyme unfoamed	PLA + 5% Enzyme unfoamed	PHA unfoamed
1	4.94	5.24	5.3	4.48	3	5.02	1.44	1.44	1.57	1.48
2	4.9	5.11	5.17	4.51	2.79	4.83	1.44	1.41	1.54	1.41
3	4.9	5.1	5.36	4.54	2.96	4.94	1.45	1.4	1.5	1.41
4	4.95	5.3	5.33	4.69	3.04	4.95	1.44	1.43	1.52	1.3
5	4.9	5.13	5.23	4.39	N/A	N/A	N/A	N/A/	N/A	N/A
Avg.	4.918	5.176	5.278	4.522	2.947 5	4.935	1.4425	1.4425	1.5325	1.4

Table 2 Material and Samples in Experiment

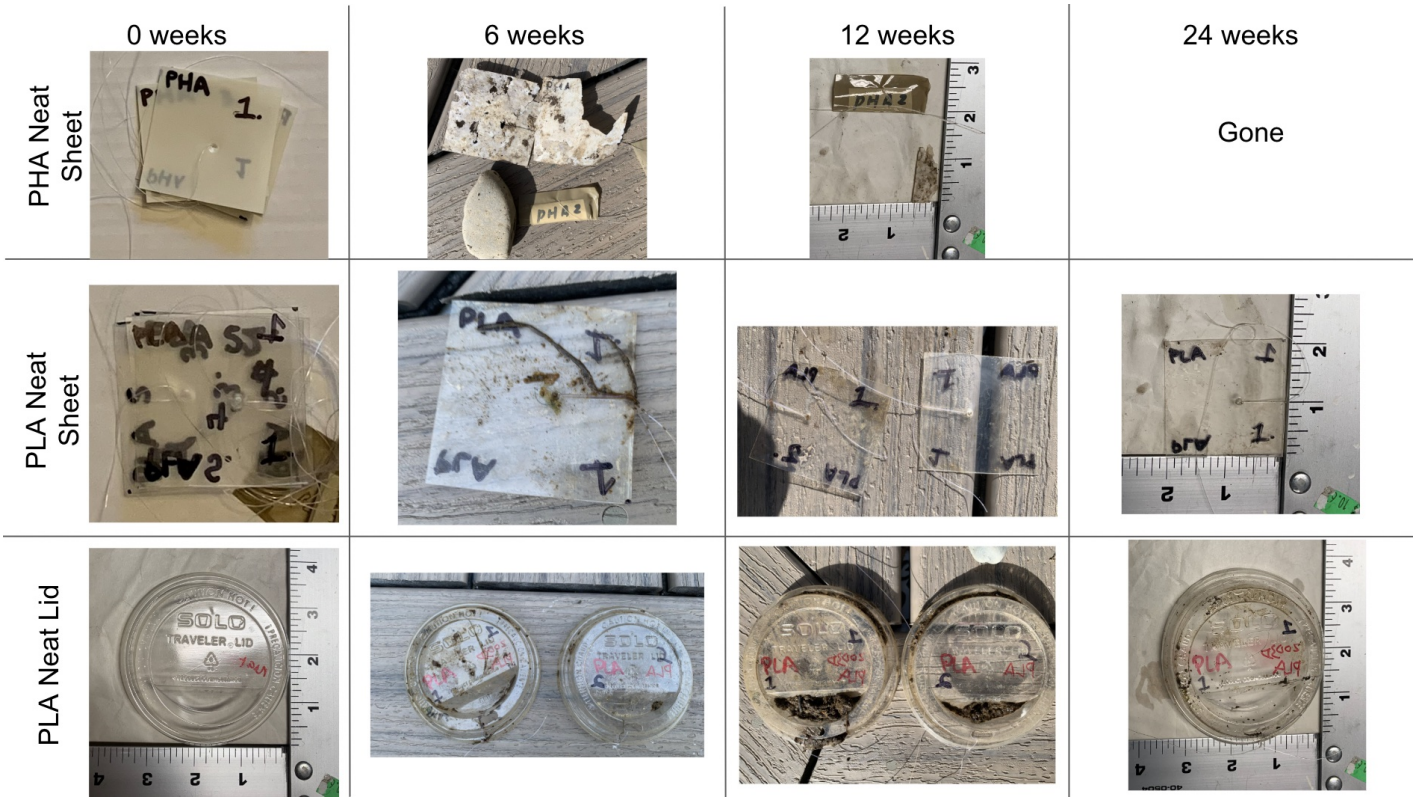


Fig. 1 100% neat PLA lids and sheets - backyard home composting result over 24 weeks, in comparison with PHA sheet.

the 2'x2" sheet samples with the same material composition in the same environment and time frame.

Figure 4 demonstrates composting results for foamed and crystallized lids, one made from PLA with 5% enzymes, and the other from neat PLA. It is believed that the foamed samples should be disintegrated and degraded faster than the solid or unfoamed samples, the reason is that the foamed samples have lower mass and lower volume-to-thickness ratio than the solid counterparts due to the amount of air uniformly distributed and presented inside the samples. On the other hand, it is expected that the crystallization may reduce the degradation rate, due to the tighter and stronger crystal structure. It was observed that the PLA with 5% enzyme lids were broken and cracked, although not as fast as the solid lid made of PLA with 5% enzyme, they

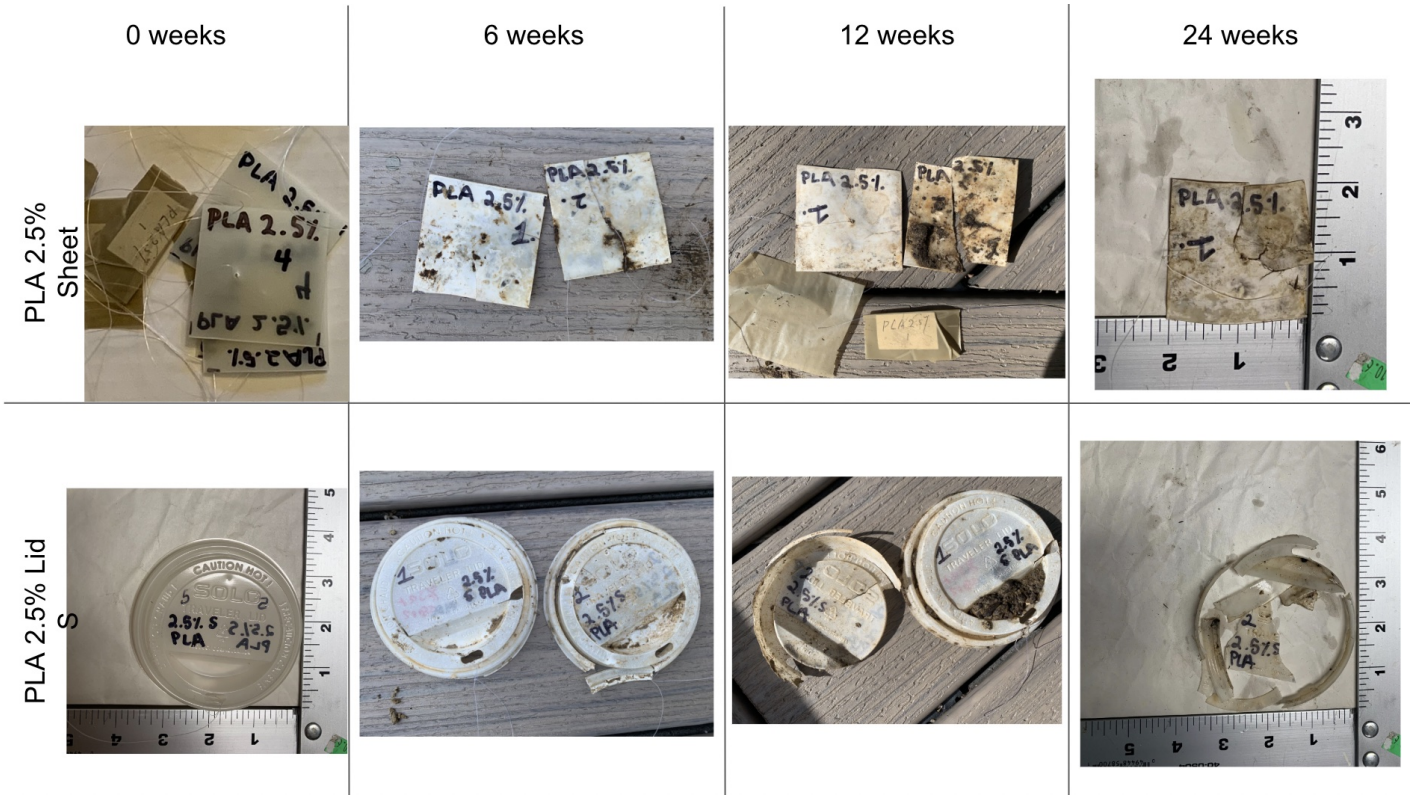


Fig. 2 PLA with 2.5% enzyme lids and sheets - backyard home composting result over 24 weeks.

had over 70% of the samples disintegrated after 24 weeks of composting, while the foamed neat PLA samples stayed intact without any sign of degradation, similar to the solid neat PLA samples.

Analysis and Discussion

Biodegradable polymers can degrade either by hydrolysis (without the enzyme catalysis) or by enzymatic mechanism. PLA is a hydrophilic and polar polyester, containing covalent ester bonds on its polymer main chain. The mechanism of the enzymatic degradation of PLA is based on the bonding of the enzyme molecules to the substrate of the PLA and then subsequently catalyzing the hydrolysis of its polymeric structure. The hydrolysis of the PLA molecules occurs by cleavage of the ester bonds in the polymer chain, leading to a decrease in molecular chain length and, thus molecular weight [11]. Each ester group hydrolyzed in the PLA chain generates a corresponding carboxylic and hydroxylic acid end group that may improve the hydrophilicity of PLA, since the occurrence of free carboxylic acid end groups in the PLA polymer matrix accelerates the hydrolysis by autocatalysis and renders the polymer matrix more polar which results in higher water uptake, then further produces smaller soluble molecular products like oligomers and lactic acid monomers that can leave PLA matrix by diffusion, resulting in mass loss [7]. This experiment aims to increase the biodegradation and disintegration rate of PLA making it home-compostable. The fundamental of this experiment is that PLA-degrading enzymes must belong to hydrolases capable of a catalytic hydrolysis reaction [12,13]. Such enzymes capable of hydrolyzing PLA include lipase, esterase, pronase, and alcalase. Evanesto® enzymes from the family of hydrolases is a polypeptide, a protein substance containing a length of amino acids, with polyester degrading activity [12–15]. The sufficient presence of these enzymes will catalyze the hydrolytic cleavage of the ester bond backbone (-COOR) in the presence of moisture or water. The composting hypothesis is that the enzymatic breakdown of chemical bonds will result in elevated disintegration and biodegradation rates [11,16]. PLA will then be degraded into low-molecular-weight oligomers, dimers, and monomers, and finally mineralized to CO2, H2O, and methane (CH4), like PHA does [17,18]. Because enzymes are proteins, they are denatured by heat. The thermostability and activity of enzymes is typically below the glass transition temperature (50-60°C) of PLA. Therefore, at higher temperatures (over about 55°C) there is a rapid loss of degrading activity as the enzyme protein suffers irreversible denaturation. Evanesto® is encapsulated enzymes that are specially engineered and optimized to withstand up to

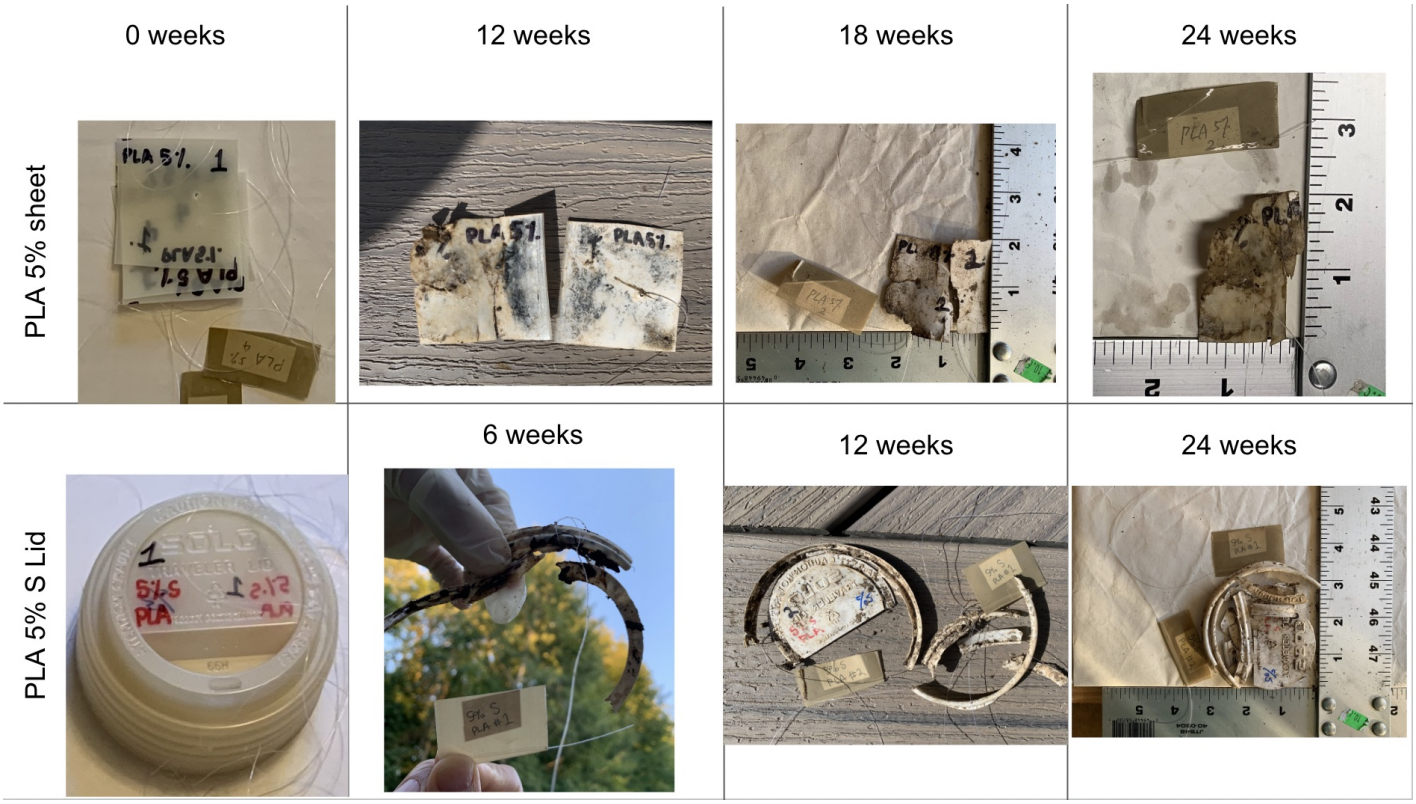


Fig. 3 PLA with 5% enzyme lids and sheets - backyard home composting result over the course of 24 weeks.

175°C extrusion temperature and heat of molten state PLA during the scalable industrial process. Embedding the enzyme into PLA was expected to accelerate the bulk erosion rate and help break PLA backbone down fully and quickly in home compost or natural conditions under ambient temperatures along with many microorganisms in the environment.

To further prove the hypothesis, the data measured and collected throughout a composting period of 24 weeks were analyzed and compared. The trends were shown and differentiated as graphed in the following charts. A clear relation between enzyme concentration within the sample and the disintegration rate by weight percentage is visible in Figure 5, which shows the relationship between the disintegration rates of PLA lid and PLA 2"x2" sheets and the concentration of enzymes. The two plotted lines clearly show that the more enzymes were within the samples, the higher the disintegration rate was, and this can be explained by the graph in Fig. 6 which indicates that the measured activity of 5% Evanesto enzymes was much higher than 2.5% enzymes, resulting in higher and faster depolymerization rate and percentage. Fig. 5 also showcases the differences between the lids and the sheets – the lids tended to degrade faster and more than the sheets due to increased surface area and thinner wall/moat thickness, even though they had the same percentage of enzyme added. This is evident with both the 2.5% and 5% lids vs. sheets.

The graph and data in Fig. 7 describe and compare the overall disintegration rates among various types of samples (Neat PLA, PLA with 2.5% enzymes, PLA with 5% enzymes, and PHA lids and sheets). The graph clearly shows the rise in disintegration rate while progressing through the samples, starting from neat PLA, with barely any biodegradability properties, to PHA, one of the most biodegradable plastics. It is conclusive how big of an impact the enzymes had on PLA, as the disintegration rate of PLA was able to increase by 35-40% with only 2.5% enzymes and by 50-85% with 5% enzymes.

The chart in Fig. 8 examines the disintegration rates between PLA with 5% enzyme lids of the unfoamed and foamed samples. The hydrolysis rate of PLA depends on a lot of factors including moisture, temperature, pH and properties such as molecular weight, density, crystallinity, etc. Crystallinity is an order within the molecular level of a polymer, and PLA can consist of amorphous and crystalline regions. Hydrolysis occurs preferentially in the free amorphous parts of the PLA matrix and the crystalline domains can only be hydrolyzed from the edges[7]. Therefore, a higher crystallinity will usually lead to a lower disintegration rate, as the polymer becomes harder to degrade. In this scenario, the foamed samples had higher crystallinity of 15.4% than the unfoamed samples with 2.91% crystallinity,

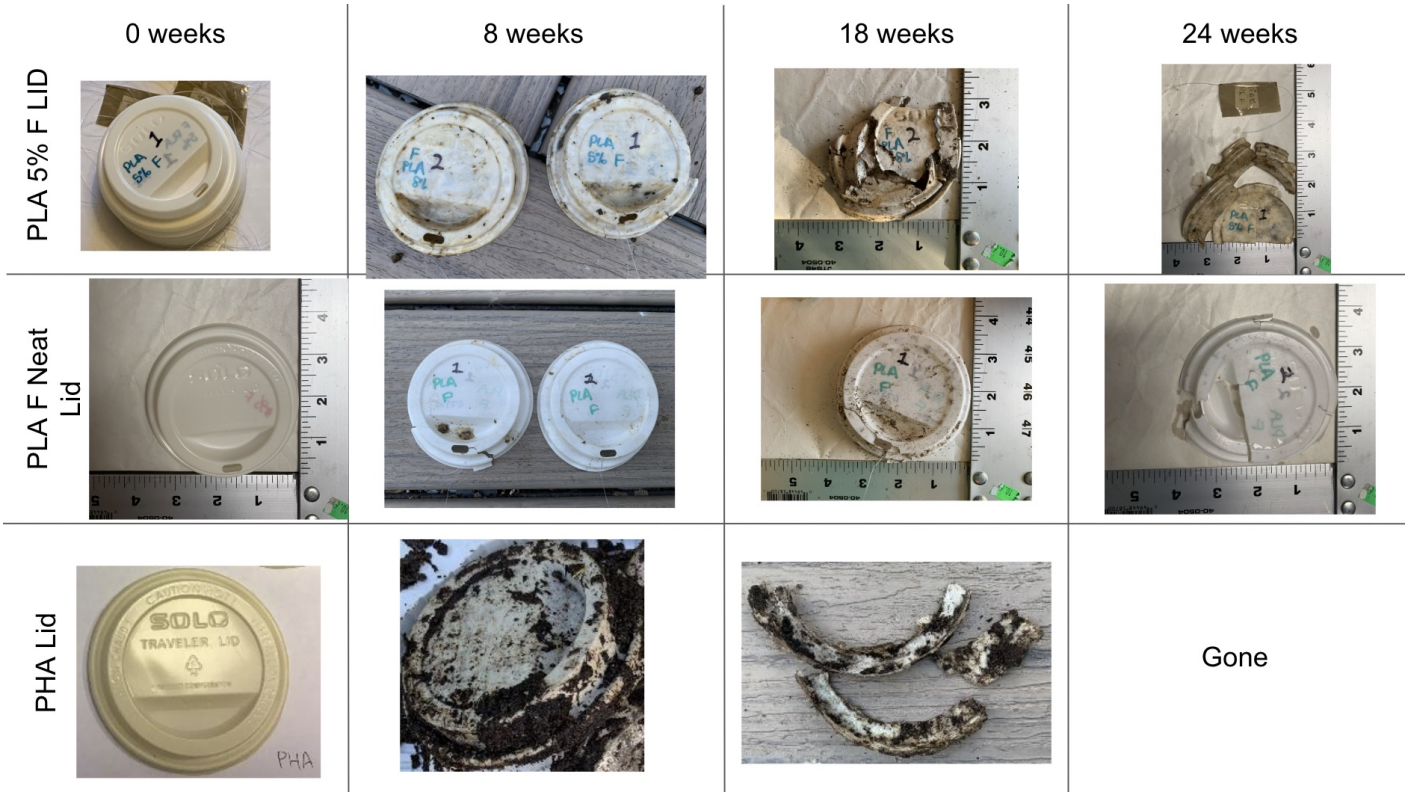


Fig. 4 Foamed and crystallized PLA lids with 5% or without enzymes- backyard home composting result over the course of 24 weeks, in comparison with PHA lid.

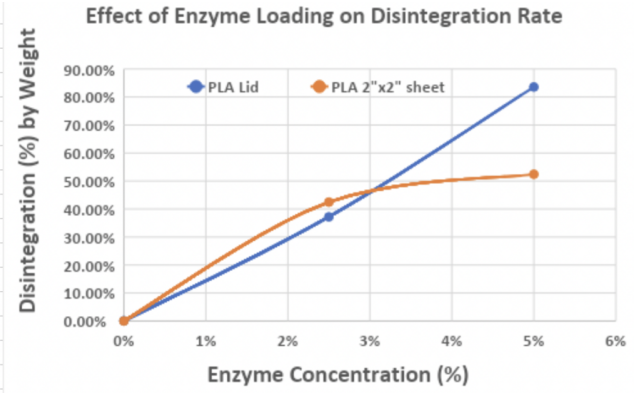


Fig. 5 Effect of enzyme concentration loading on disintegration rate of PLA lid and sheet

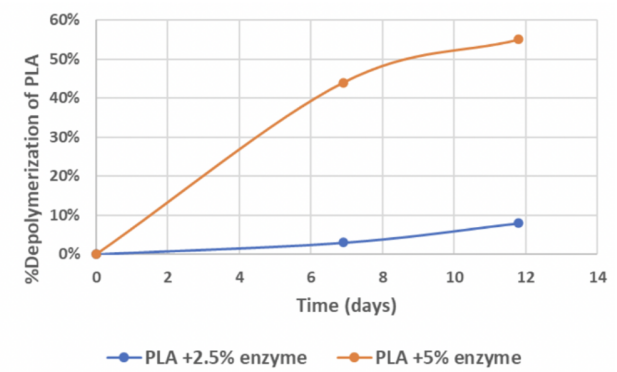


Fig. 6 Evanesto® enzyme activity in each PLA sheet and lid sample at the condition of 28°C, pH9

due to the molecules being stretched by air bubbles, so there are more order and aligned polymer chains in the foamed lids, which also had a lower density of 0.66 g/cm3 than the unfoamed lid (1.27 g/cm3). The graph indicates that the unfoamed lid had a higher disintegration rate of 83.57%. In this case, however, due to the foamed lid having higher crystallinity, even though it has lower density, the higher crystallinity wins over the lower density in controlling the disintegration rate of the foamed lid, which became lower at 53.50%. This can be seen in Fig. 5, as at the end of the 24 weeks, more foamed lid parts remained compared to the unfoamed lids.

This graph in Fig. 9 shows the differences in surface erosion of the samples at the end of the composting of 24 weeks. Surface erosion was measured with a percent change in the thickness of the initial samples and at the end of the experiment. From the chart, visible differences between the PLA with enzyme

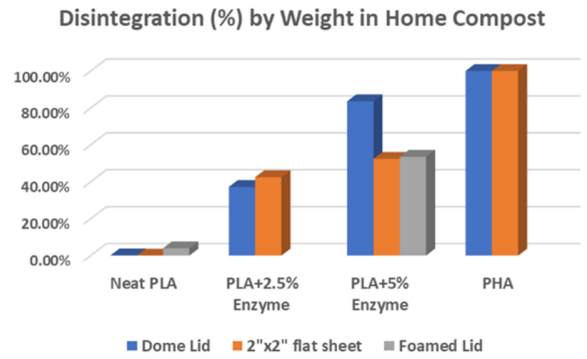


Fig. 7 Disintegration (%) by weight of different samples in home compost

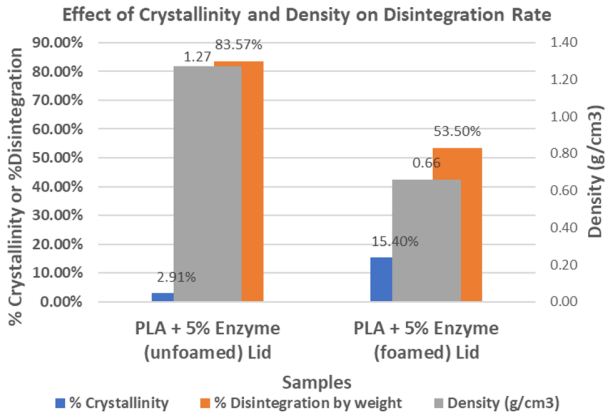


Fig. 8 Effect of crystallinity and density on disintegration rate of PLA samples with 5% enzyme

samples and neat PLA samples are shown. The higher the enzyme concentration was, the more surface erosion occurred. This was more visible on the sheet samples than on the lids. However, compared to PHA, the surface erosion on PLA with enzyme samples was still much lower. The data suggested that the biodegradation of PLA with enzymes may be controlled by overall bulk breakdown, while PHA biodegradation is dominated by surface erosion. This is because hydrolysis affects all ester groups in the whole polymer matrix leading to hydrolytic degradation throughout the whole PLA substrate in a process of bulk erosion. Although PLA is a polar polyester material, the surface of PLA still lacks sufficient hydrophilic functional groups for fast hydrolysis. On the other hand, for enzymes to be active in PLA surface erosion, microbes in the compost need to encounter and colonize the PLA surface, physically attach and chemically sense the PLA substrate. When signaling molecules have reached a threshold concentration, they induce microbial production and secretion of the PLA-degrading enzymes onto the PLA surface, such that the enzyme's active site is in proximity to ester groups in PLA chains [10]. Unlike microbial-synthesized PHA, extracellular PHA-degrading enzymes produced by microorganisms are widespread across the environment, and chemically synthesized PLA has no or very limited dedicated degrading enzymes to induce surface erosion.

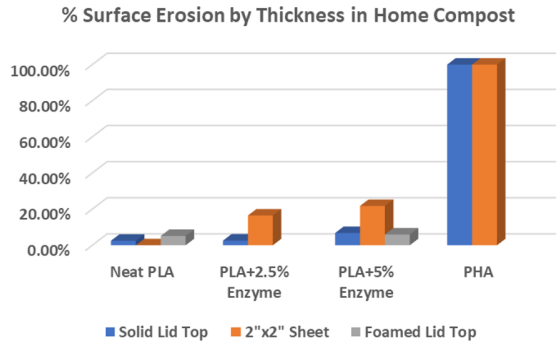


Fig. 9 % Surface erosion by the thickness of different samples in home compost

Figures 10-11 present DSC (differential scanning calorimetry) and TGA (thermogravimetric analysis) results for the PLA lids made of different concentration levels (at a) 0%, b) 2.5%, and c) 5%) of the enzyme before and after 6 months (or 24 weeks) of biodegradation under compost conditions. The DSC results in Fig. 10 point out some changes in the thermograms (glass transition, crystallization, melting temperatures, crystallinity, and heat of fusion) between pre-composting and 6-month post-composting samples of each lid that was made with or without the addition of enzymes. For both PLA with 2.5% and 5% enzyme, after 6 months of compost biodegradation, the percentage of crystallinity was slightly increased probably due to the shortening of chain length from the ongoing degradation, leading to several-degree rises in glass transition temperature. The TGA results in Fig. 11 indicate a decrease in onset degradation temperatures for PLA samples with enzymes after 24 weeks of incubation in compost was observed in comparison to that unchanged for neat PLA. These analyses further show that the Envesto®enzyme accelerated the biodegradation process of PLA compared to the neat polymer. Figure 12 recorded CO2 production over time of 6 months for various PLA lids embedded with different enzyme concentrations at 0%, 2.5% and 5% in comparison to the cellulose blank as reference. The PLA 2003D with 5% enzyme lids demonstrated biodegradation under home compost conditions based on EN 13432 or ASTM D6400 Standard since over 90 percent (92.29%) of the carbon in the PLA lid with 5% enzyme was converted to CO2 after 180 days in the test, while PLA lid with 2.5% enzyme had 67.43% carbon and neat PLA lid had 54.27% carbon converted to CO2 at the end of 6 months. Further, the foamed PLA lid with 5% enzyme, which had higher crystallinity than the unfoamed PLA lid with the same amount of 5% enzyme, converted less than 90% (79.77%) of the carbon in the PLA lid to CO2, due to the impact of crystallinity. The cellulose control reference had 82.37% car-

bon converted to CO2 during the same test period. Nevertheless, the data and graphs clearly demonstrated and proved that the encapsulated enzyme has indeed increased not only the disintegration rate but also the biodegradation rate of the PLA articles. It is believed that the embedded enzyme in the PLA substrate and thus formed microstructure at a macromolecular level makes small holes, clefts and cavities from the inside, increasing the surface area of the PLA products and enabling microbes and microorganisms to attach to the newfound cavities. This thus accelerates the biodegradation rate in natural conditions after use by rapidly breaking down the PLA into smaller pieces.

Conclusion and Future Work

The experiments included testing and investigation of biodegradability and home compostability of modified PLA lids using Evanesto® enzymes based on ASTM and ISO test standard methods as reference. PLA requires temperature at its softening temperature (50-60°C) in industrial compost to degrade. However, Evanesto® enzymes can accelerate the disintegration and biodegradation rate of PLA under backyard composting conditions, thus enabling PLA home compostability and allowing PLA to expand its horizons as a possible alternative for alleviating microplastic pollution. With the 5% concentration of enzymes in PLA, an average of about 85% of the PLA lids by weight were disintegrated and over 92% or the carbon in the PLA substrate was converted to CO2 after 6 months of composting, and certain samples even completely biodegraded like PHA samples did. The higher the enzyme concentration in PLA, the faster the disintegration rate. With optimized enzyme concentration at a slightly increased percentage and process, it was expected that the enzyme embedded PLA could be disintegrated at a higher rate of >90% under backyard composting conditions in 24 weeks to meet home-compostable standard. Furthermore, the biodegradation rate was faster with a lower mass volume to surface ratio, thus showing lower density helps increase the degradation rate due to the reduced amount of PLA mass. However, higher crystallinity reduces the compostability. Surface erosion was much less significant on the PLA samples with the enzymes than on PHA samples. This research study has demonstrated that like PHA, the enzymatically modified PLA, with properly engineered and optimized enzymes, having all necessary characteristics of fossil-based commodity plastics, such as PP, can be rapidly composted and biodegraded in nature after use to yield CO2, H2O, etc. at the end of its life leaving no residues of microplastics, this is in agreement with the finding HYDRA Marine Sciences reported that in the presence of water or humidity and microorganisms, when PLA is fully hydrolyze, and no persistent nano- or microplastics will remain or accumulate in the environment[7]. This study provides a new strategy to modify and prepare biodegradable and compostable plastics that can be degraded under natural conditions

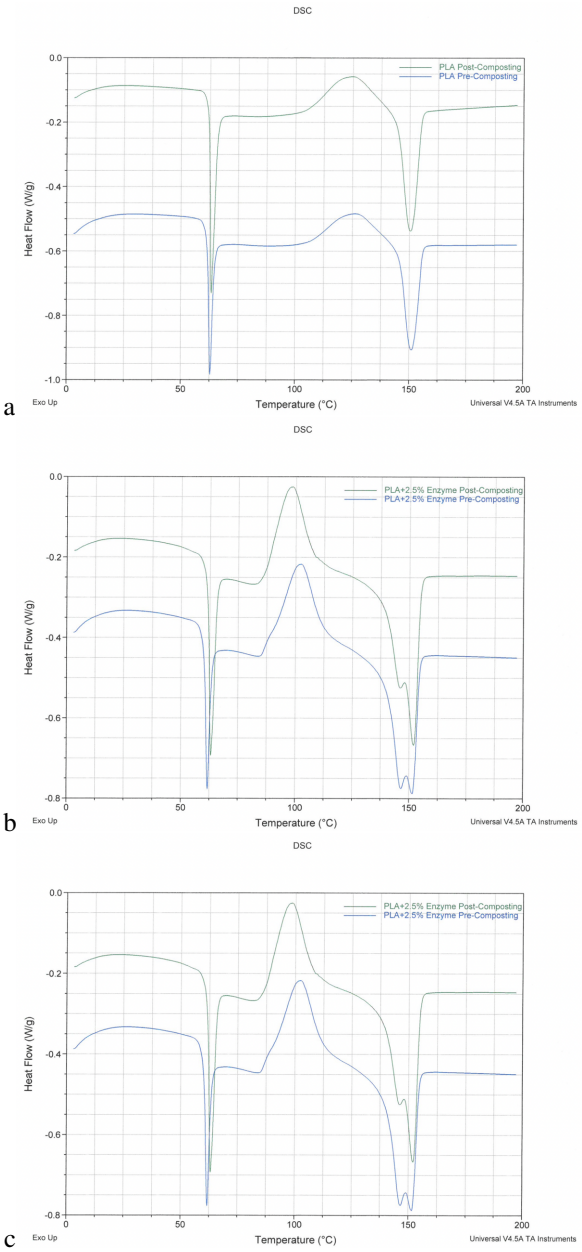
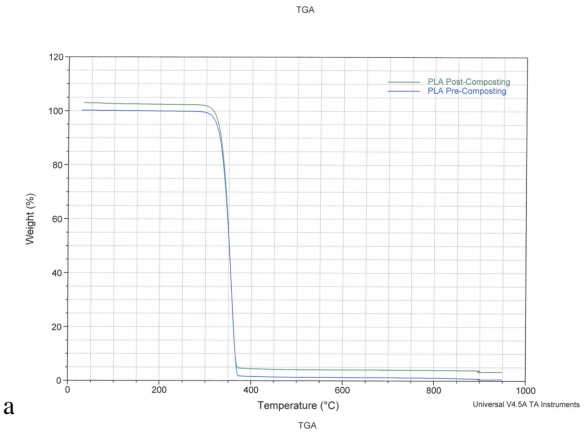
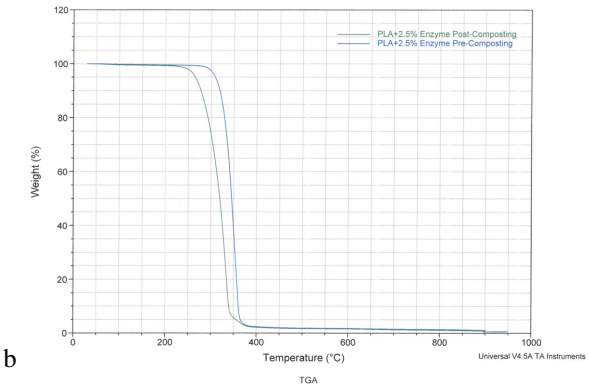


Fig. 10 DSC analysis of (a) PLA with 0% enzyme (b) PLA with 2.5% enzyme (c) PLA with 5% enzyme lids before and after 6 months of biodegradation under compost conditions

with fewer and no microorganisms. The novelty of this research is enlightened by the thermal stability and effectiveness of the enzymes encapsulated in PLA, further demonstrated through the significant findings that the embedded enzymes can be applied to extrusion thermoforming processing, produce and result in PLA products that can have faster biodegradation and higher disintegration rate in nature environment to achieve home compostability and thus reduce microplastics pollution. Therefore,



a



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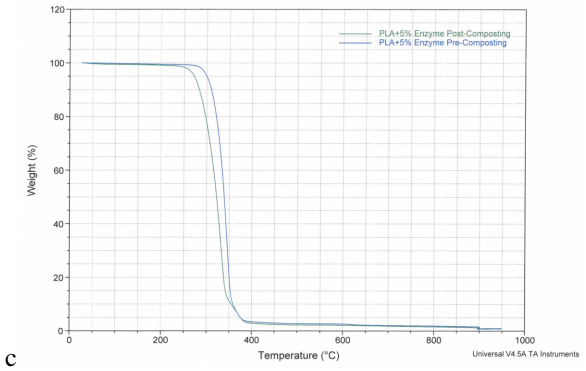

c

Fig. 11 TGA analysis of (a) PLA with 0% enzyme (b) PLA with 2.5% enzyme (c) PLA with 5% enzyme lids before and after 6 months of biodegradation under compost conditions

instead of directly banning all plastics, biodegradable and compostable plastics should be considered, offered more widely, and used more extensively to replace petrochemical plastics, especially for thin gauge single-use food packaging applications such as coffee or drinking paper cups and lids, which are not normally collected and recycled in the recycling industry. The composting process is particularly appropriate for dealing with food-contaminated packaging, as recycling facilities cannot deal

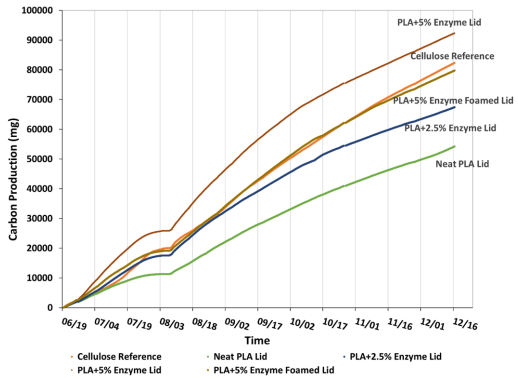

Fig. 12 CO_2 production over time of 6 months for various PLA lids embedded with different enzyme concentrations (0%, 2.5% and 5%) and cellulose blank reference.

with food-contaminated plastics, at the same time the compost formed can be used for soil improvement.

For future work, the focus will be to optimize the enzyme concentration for high crystalline PLA in a hot service application, scale up, and test the optimized composition in a manufacturing plant under production equipment and conditions. Another aspect to expand would be the testing of these samples in various marine environments, including freshwater and ocean water. Advocacy for lower prices and more commercial manufacturing for this novel discovery is also important for utilizing this technology and improving the path of fighting microplastic pollution.

References

1 E. Program, *Our Planet is choking on plastic*, https://www.unep.org/interactives/beat-plastic-pollution/.

2 V. Beghetto, R. Sole, C. Buranello, M. Al-Abkal and M. Facchin, *Recent Advancements in Plastic Packaging Recycling: A Mini-Review*, 2021, https://doi.org/10.3390/ma14174782.

3 J. Hwang, D. Choi, S. Han, S. Y. Jung, J. Choi and J. Hong, *Potential toxicity of polystyrene microplastic particles*, 2020, https://doi.org/10.1038/s41598-020-64464-9.

4 G. X. Wang, D. Huang, J. H. Ji, C. Völker and F. R. Wurm, *Seawater-Degradable Polymers—Fighting the Marine Plastic Pollution*, 2021, https://doi.org/10.1002/advs.202001121.

5 F. Buijzen, F. Bie, J. De and Lovett, *End-of-life options for bioplastics (V1.0)*, www.total-corbion.com.

6 F. Buijzen, M. Ravard, P. Scola, R. L. Scola and R. Veras, *The advantages of compostable bioplastics for the circular economy*, https://www.totalenergies-corbion.com/.

7 C. Lott, K. Stemmer-Rau and M. Weber, *A meta-study on the persistence and toxicity of PLA, and the formation of microplastics in various environments*, Technical Summary Report, Hydra Marine Science, Buhl, Germany, HYDRA Marine Sciences GmbH - STARTSEITE International.

8 A. Shalem, O. Yehezkeli and A. Fishman, *Enzymatic degradation of Polylactic acid (PLA)*, 2024, https://doi.org/10.1007/s00253-024-13212-4.

9 W. A. Ahsan, A. Hussain, C. Lin and M. K. Nguyen, *Biodegradation of Different Types of Bioplastics through Composting—A Recent Trend in Green Recycling*, 2023, https://doi.org/10.3390/catal13020294.

10 M. Huaman *et al.*, *A general route to retooling hydrolytic enzymes toward plastic degradation*, 2024, https://doi.org/10.1016/j.xcrp.2024.101783.

11 E. Urinov, S. Hanstein and A. Weidenkaff, *Enzymatic Degradation of Fiber-Reinforced PLA Composite Material*, 2022, https://doi.org/10.3390/macromol2040033.

12 J. Kaushal, M. Khatri and A. Shailendra, *Recent insight into enzymatic degradation of plastics prevalent in the environment: A mini - review*, 2021, https://doi.org/10.1016/j.clet.2021.100083.

13 N. Mohanan, Z. Montazer, P. K. Sharma and D. B. Levin, *Microbial and Enzymatic Degradation of Synthetic Plastics*, 2020, https://doi.org/10.3389/fmicb.2020.580709.

14 D. Silva, M. Kaduri, M. Poley, O. Adir, N. Krinsky, J. Shainsky-Roitman and A. Schroeder, *Biocompatibility, Biodegradation and Excretion of Polylactic Acid (PLA) in Medical Implants and Theranostic Systems*, 2018, https://doi.org/10.1016/j.cej.2018.01.010.

15 M. E. E. Temporiti, L. Nicola, E. Nielsen and S. Tosi, *Fungal Enzymes Involved in Plastics Biodegradation*, 2022, https://doi.org/10.3390/microorganisms10061180.

16 C. Maraveas, M. I. Kotzabasaki and T. Bartzanas, *Intelligent Technologies. Enzyme-Embedded and Microbial Degradation of Agricultural Plastics*, 2023, https://doi.org/10.3390/agriengineering5010006.

17 *4 major solutions to fight plastic pollution*, Carbiolice, April 5, https://www.carbiolice.com/en/news/4-solutions-to-fight-plastic-pollution/, 2021.

18 K. W. Meereboer, M. Misra and A. Mohanty, *Review of Recent Advances in the Biodegradability of Polyhydroxyalkanoate (PHA) Bioplastics and Their Composites*, 2020, https://doi.org/10.1039/D0GC01647K.